A FIRST REPORT ON MYCORRHIZA ASSOCIATED WITH SOME TRANSVAAL PLANTS.

By C. Cohen.

(*Department of Botany, University of the Witwatersrand.*)

(With Plate X.)

Mycorrhizal association with a variety of plants in different families, in varying ecological situations, has been widely reported from all over the world, but no systematic investigation has been reported from this country.

Particular interest in the occurrence of mycorrhizas in the Transvaal was stimulated by reports of their presence in the roots of local grasses and herbs (1, 4). The writer, however, has not been able to find a fungus of the mycorrhizal type in any of the plants mentioned by these authors which he checked. Undoubtedly, examination of apparently quite normal roots will often show the presence of fungal threads, but as indicated below, microtomed sections with careful techniques are recommended, whereas Page and Coetzee report a wealth of detail from hand-cut sections stained with cotton blue.

The following methods were examined for the testing of fresh material :—

Staining with Pianese 3B, differentiating with acid alcohol, and clearing with benzene phenol.

Margolena's modification of Stoughton's method, using 0·1% thionin in 5% aq. phenol, counterstaining with 0·5% light green in alcohol, and Orange G and erythrosin in clove oil.

Staining with 0·1 g. Ruthenium red in 15 cc. distilled water, differentiating in KOH.

Iron haematoxylin counterstained with erythrosin.

Mordanting an entire root in 2% iron alum, staining in haematoxylin, differentiating in acid alcohol, and counterstaining with Orange G.

Clearing in lactophenol, staining in cotton blue in lactic acid, and differentiating in lactic acid.

While the first, second, and last methods were good, it was soon obvious that hand sectioning was unsatisfactory for mycorrhizal examination, and was often extremely difficult, for anatomical reasons, with some grasses.

The following combinations of fixatives and stains were then tried on microtomed material, with every possible pairing :—

Fixatives. Helly fluid ; formalin-aceto-alcohol ; chromacetic, weak and strong ; Carnoy's ; Flemmings ; 5% chromic sulphate in saturated picric or salicylic acids (2).

Stains. Iron haematoxylin with fuchsin iodine ; Ikata's method (3) ; iron haematoxylin with Pianese 3B ; safranin and fast green or a triple combination ; Benda's haematoxylin ; Margolena's method ; Pianese 3B ; lactophenol and cotton blue ; Conant and Johansen's quadruple stains (3).

Helly fluid with Conant's quadruple stain was finally selected, and the following method employed :—

Material was fixed overnight in Helly fluid, made as below :—

Distilled water, 100 cc. ; Mercuric chloride 5 g. ; Potassium dichromate, 2·5 g. ; Sodium sulphate, 1 g. To 20 cc. of this fluid, 1 cc. of neutralized 40% formalin was added immediately before use.

The material was then washed in water, and the mercury removed before cutting by $\frac{1}{2}$% iodine in 70% alcohol. The iodine was removed by washing the material with 70% alcohol, followed by 5% sodium thiosulphate, which was then removed by washing with water.

The material was next passed through 30% and 50% ethyl alcohol, and through the following mixtures, each of these steps lasting about two hours :—

Distilled water, 50 cc. ; 95% ethyl alcohol, 40 cc. ; butyl alcohol, 10 cc.
Distilled water, 30 cc. ; 95% ethyl alcohol, 50 cc. ; butyl alcohol, 20 cc.
Distilled water, 15 cc. ; 95% ethyl alcohol, 50 cc. ; butyl alcohol, 35 cc.
95% ethyl alcohol, 45 cc. ; butyl alcohol, 55 cc. ; butyl alcohol, 75 cc.
absolute ethyl alcohol, 25 cc.

The material was thereafter passed through three changes of pure butyl alcohol, the second or third being overnight, and then left for two or more hours in a mixture of butyl alcohol and paraffin oil. It was then passed through two changes of Parowax, and finally embedded in 52—54° C. paraffin wax.

The blocks were poured, cooled in water, and kept until used in a refrigerator.

Before cutting, the face of the block was exposed with a sharp razor, and the block was soaked in iced distilled water for 1—2, or 12, or a maximum of 24 hours.

Sections were cut at between 8—10, and up to 30 mu.

Haupt's adhesive, made as follows, was used for fixing sections, to slides :—

1 g. of pure, plain gelatin was dissolved in 100 cc. distilled water at 30° C. When completely dissolved, 2 g. phenol crystals were added and 15 cc. glycerine. In very cold weather, it was advisable to keep the adhesive in an incubator at below 30° C. ready for use.

The sections were floated on a bath of 4% formalin, which helped to stretch them, and then lifted from the bath on a prepared slide. They were allowed to dry, without further draining. If the sections were very thick, it was necessary to keep the slides constantly exposed to formalin vapour for up to six hours, at room temperature, to harden the adhesive.

When the sections and slides were completely dry, they were passed through pure xylol, then a mixture of absolute alcohol and xylol, in each case, for 5 minutes. They were then left in 95% ethyl alcohol, and 70% alcohol, for 2 minutes. They were then stained for 2 hours or more in 1% safranin in 50% alcohol. The sections were then washed in water, stained in 1% aq. crystal violet for about a minute, washed in water and two changes of absolute alcohol, before being stained for 1 minute in 0·1% alcoholic Fast Green FCF. This was followed by three transfers through Orange G in clove oil, and a wash in xylol, before being mounted in Clarite.

Polypodiaceae.
- Pteridium aquilinum. Negative.

Commelinaceae.
- Cyanotis nodiflora. Negative.

Gramineae.
- Andropogon appendiculatus. Negative.
- Aristida junciformis. Negative.
- Avena sativa. Negative.
- Brachiaria serrata. Negative.
- Eragrostis capensis. Negative.
- Eragrostis chloromelas. Negative.
- Eragrostis nebulosa. Negative.
- Helictotrichon cf. turgidulum. Negative.
- Hordeum vulgare. Negative.
- Hyparrhenia hirta. Negative.
- Lolium perenne. Probable, tiller leaves ; negative, roots, stems and upper leaves.
- Rhynchelytrum repens. Negative.
- Secale cereale. Negative.
- Tristachya hispida. Negative.
- Triticum sativum. Negative.
- Zea Mays. Negative.

Liliaceae.
- Aloe transvaalensis. Negative.

Amarantaceae.
- Alternanthera repens. Negative.
- Gomphrena globosa. Pelotons.

Casuarinaceae.
- Casuarina Cunninghamiana. Negative. Pericarp wing and seed coat.

Compositae.
- Berkheya speciosa. Pelotons.
- Berkheya subulata. Negative.
- Bidens Schimperi. Negative.

Conyza ambigua. Arbuscule-sporangiole.
Conyza ivaefolia. Negative.
Cosmos bipinnata. Negative.
Dicoma Zeyheri. Arbuscule-sporangiole.
Geigeria Burkei. Negative.
Galinsoga parviflora. Arbuscule-sporangiole.
Haplocarpha scaposa. Arbuscule-sporangiole.
Helichrysum cf. leiopodium. Negative.
Helichrysum Cooperi. Arbuscule-sporangiole.
Inula graveolens. Arbuscule-sporangiole.
Nidorella hottentotica. Pelotons.
Nidorella polycephala. Pelotons.
Schukhria bonariensis. Negative.
Senecio coronatus. Negative.
Senecio fraudulentus. Pelotons.
Senecio glandulifera. Pelotons.
Senecio hydrorhizus. Arbuscule-sporangiole.
Senecio isatideus. Negative.
Senecio laevigatus. Negative.
Senecio serratuloides. Negative.
Senecio succosus. Negative.
Senecio venosus. Negative.
Tagetes minuta. Negative.
Ursinia annua. Negative.
Vernonia Kraussii. Negative.
Xanthium pungens. Negative.
Xanthium spinosum. Arbuscule-sporangiole.

Cruciferae.
Brassica rapa. Negative.
Lepidium bonariensis. Negative.
Nasturtium officinale. Negative.

Dipsaceae.
Scabiosa anthemifolia. Pelotons.

Euphorbiaceae.
Acalypha caperonoides. Negative.
Euphorbia platyphylla. Negative.

Loganiaceae.
Gomphostigma virgata. Negative.

Leguminosae.
Cassia minosoides. Negative.
Medicago sativa. Negative.
Robinia pseud-acacia. Negative.
Sphenostylis angustifolia. Negative.
Trifolium repens. Negative.
Vicia sativa. Negative.
Vigna vexillata. Negative.

Malvaceae.
Hibiscus malacospermus. Negative.
Hibiscus trionum. Arbuscule-sporangiole.

Polygonaceae.
Polygonum glutinosum var. capense. Negative.

Rosaceae.
Prunus persica. Negative.

Rhamnaceae.
Zizyphus mucronata. Negative.

Solanaceae.
Datura stramonium. Arbuscule-sporangiole.
Physalis minima. Negative.
Solanum capense. Negative.
Solanum panduraeforme. Negative.
Solanum tuberosum. Negative.

Tropaeolaceae.
Tropaeolum majus. Negative.

Umbelliferae.
Daucus carota. Negative.

Verbenaceae.
Verbena bonariensis. Negative.
Verbena erinoides. Negative.

Discussion and Summary.

The number of families, genera or species examined was not large in number, so that it is difficult to draw definite conclusions from the finding that 18 out of 82 species contained a fungus of the mycorrhizal type. Moreover, with the exception of some maize plants, and the single example of a fern, all the plants examined came from the Witwatersrand area, that is, from a sandy soil of granite origin.

The two morphological types were not confined to any family or genus, nor were they seen together, in any plant.

Only one member of the Gramineae, of those examined, showed a mycorrhiza.

Mycorrhizas were not confined to perennial plants.

No mycorrhiza was found in any crop plant, although such cases have been reported from overseas.

A relatively large proportion of those " weeds " examined showed a mycorrhiza.

Acknowledgments.

My best thanks are due to Prof. J. F. V. Phillips, in whose department this work was done, for suggesting the subject to me, and for his interest since ; and to Mr. S. W. Adamson, who, by collecting the plants for examination, made the work possible.

BIBLIOGRAPHY.

1. Coetzee, J. A first contribution to the ecology of certain high-veld communities ; some subseral and priseral types. Unpublished M.Sc. thesis. University Witwatersrand. 1945.

2. Cohen, I. and Doak, K. The fixing and staining of *Liriodendron tulipifera* root tips and their mycorrhizal fungus. Stain. Tech. 10. 1. 25—32. 1935.

3. Johansen, D. Plant microtechnique. New York. 1940.

4. Page, M. A first contribution to the root ecology of certain high-veld communities ; lower secondary successional stages (subsere). Unpublished M.Sc. Thesis. 1945.

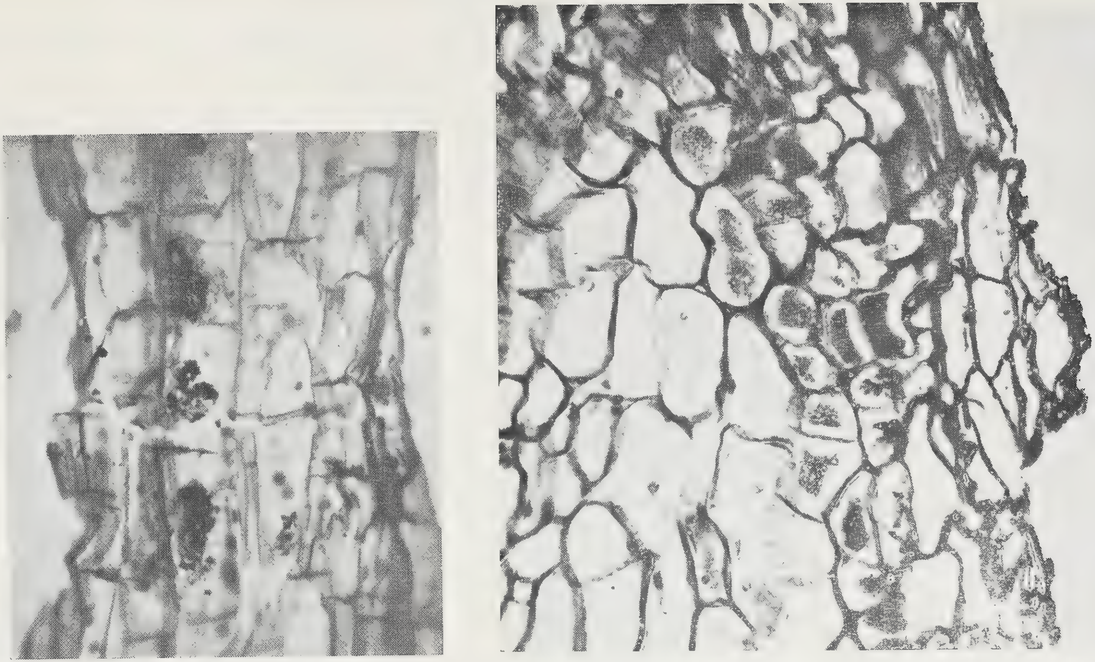

Plate X. Mycorrhiza.

(From slides cut at 10 mu, and stained with Conant's Safranin-Crystal Violet-Fast Green-Orange G combination.)

1. L. S. root of *Conyza ambigua*, showing arbuscule-sporangioles. In this type, a branching structure, at this stage, bears densely staining organs, which are apparently in a process of digestion and disintegration. Sometimes, therefore, only the branching is seen, if the process has gone far. The arbuscule-sporangiole is always seen within the cell, and in this case stains densely blue-violet. It tends to lie in the cortical regions nearer the endodermis. × 185.

2. T. S. Root of *Gomphrena globosa*, showing the skeins called pelotons, which stain reddish purple. They are found in the cortical regions, and the outermost tend to show digestion. × 185.